PIPERAZINE DIHYDROCHLORIDE AND GLYCYLGLYCINE AS NON-TOXIC BUFFERS IN DISTILLED WATER AND IN SEA WATER[1,2]

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A wide selection of buffers is necessary in biological work, since it is often desirable to repeat a particular experiment with a different buffer. Piperazine dihydrochloride and glycylglycine are crystalline, non-volatile, very soluble solids readily obtainable in pure form. Piperazine is relatively non-toxic to man (Hanzlik, 1917) and to rats (Dieke, Allen, and Richter, 1947) and has been used as an apparently non-toxic buffer by certain biologists at our suggestion (Cornman, 1940, 1941; Evans, Beams, and Smith, 1941). The buffer merits of glycylglycine in sea water have been previously pointed out by Tyler and Horowitz (1937). This relatively non-toxic material is a normal constituent of many proteins. A wide-range buffer is simply and accurately prepared from only these two substances and sodium hydroxide. Used in sea water there is no observed precipitation of salts until a pH of 9.9 is reached. The commonly used phosphate buffer precipitates calcium and magnesium phosphate from sea water at a much lower pH, thus disturbing the salt balance and adding uncertainty to conclusions from experiments. The shortcomings of many of the buffers in common use have recently been mentioned by Gomori (1946). We have not used the new buffers suggested by him and cannot compare his buffers with ours, except to point out that our buffers have a wider range.

For special cases where it is desired to have no inorganic ions in a buffer, it is possible to obtain buffers from a pH of 7.0 to 11.0 by titrating glycylglycine with the free base of piperazine. However, we are presenting no data on this subject.

In this paper we present a table indicating the preparation of several buffers, using piperazine dihydrochloride, glycylglycine and equimolecular mixtures of the two substances in distilled water and in sea water. We also present pK_1 and pK_2 values of piperazine dihydrochloride.

Experimental

The piperazine was purchased from the Eastman Kodak Co. in the form of the hexahydrate. Because the free base of piperazine absorbs carbon dioxide and moisture from the air, it was converted into the stable dihydrochloride (Sieber, 1890) before use.

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Piperazine dihydrochloride is prepared by dissolving 50 g. of piperazine hexahydrate in 100 ml. of 95 per cent ethanol, and adding slowly 100 ml. of concentrated hydrochloric acid. Heat is evolved. As the mixture cools, crystals of the dihydrochloride hydrate are formed. The mixture is cooled in an ice bath and is filtered. The crystals are washed several times with cold ethanol, and are air-dried. The material is ready for use after it has been dried at 100° C. for eight hours. The yield is 33 g. Anhydrous piperazine dihydrochloride is slightly hygroscopic.

Analytically pure glycylglycine was purchased from the Amino Acid Manufactures of the University of California at Los Angeles and was used without further purification. The material was dried at 100° C. for six hours just prior to use. Glycylglycine is not appreciably hygroscopic.

The pH measurements were made at 25° C. (± 0.2) with a Leeds and Northrup potentiometer-electrometer No. 7660, equipped with Leeds and Northrup glass dip electrode Std. 1199–12 made of Corning 015 glass, and a reference saturated calomel half-cell electrode Std. 1199–13 with a potassium chloride capillary salt bridge. Before and after each titration the electrode was checked against "standard acetate," for which the pH value of 4.64 was taken (MacInnes, Belcher, and Shedlovsky, 1938). As is the practice in standardizing buffers, the liquid junction potentials were neglected.

The sodium ion error for the higher pH values was corrected by using the following equation adapted from Powney and Jordan (1937) to fit the sodium ion errors found experimentally when our glass electrode was calibrated with the hydrogen electrode.

$$\text{Log } \Delta\text{pH} = 0.50 \text{ pH} - 5.86 + 0.46 \text{ log } [\text{Na}^+]$$

The possible error in these readings increases with increasing alkalinity, but below a pH of 9.0, the accuracy was within the limits of ± 0.02.

Although stock solutions of piperazine dihydrochloride and glycylglycine may be prepared, it is preferable to prepare the solutions fresh, since on long standing glycylglycine may undergo hydrolysis and piperazine dihydrochloride might form toxic products (Greenbaum, 1937).

Solutions of piperazine and glycylglycine and equimolecular mixtures of the two were titrated with standardized sodium hydroxide and numerous readings were taken. From these readings a table for the preparation of buffers was made (Table I).

Several pH determinations were made with the glass electrode and the hydrogen electrode on solutions which were equimolecular with respect to piperazine dihydrochloride and the monohydrochloride (pK_1') and on solutions which were equimolecular with respect to the monohydrochloride and free piperazine (pK_2') (Table II).

Discussion

We have used the available data in Table II in making approximate calculations of the ionization exponents of piperazine at infinite dilution, since this has not been previously reported. Using the standard Debye-Hückel equation for moderately dilute solutions, we have found values for piperazine dihydrochloride for pK_1 of

Table I

Table for Preparation of Buffers at 25° C.

(1) 0.1591 g. piperazine dihydrochloride diluted to 100 ml. with distilled water to which is added 0.1000 N sodium hydroxide as indicated below in column (1).
(2) 1.591 g. piperazine dihydrochloride diluted to 100 ml. with distilled water to which is added 1.000 N sodium hydroxide.
(3) 15.91 g. piperazine dihydrochloride diluted to 100 ml. with distilled water to which is added 1.000 N sodium hydroxide.
(4) 0.1321 g. glycylglycine diluted to 100 ml. with distilled water to which is added 0.1000 N sodium hydroxide.
(5) 0.1591 g. piperazine dihydrochloride plus 0.1321 g. glycylglycine diluted to 100 ml. with distilled water to which is added 1.000 N sodium hydroxide.
(6) 0.1591 g. piperazine dihydrochloride diluted to 100 ml. with filtered sea water (pH 8.0) to which is added 1.000 N sodium hydroxide.
(7) 0.1591 g. piperazine dihydrochloride plus 0.1321 g. glycylglycine diluted to 100 ml. with filtered sea water (pH 8.0) to which is added 1.000 N sodium hydroxide.

Buffer pH	ml. of NaOH to be added to above solutions						
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
4.4	0.66				0.007		
4.6	1.11	0.78			0.054		
4.8	1.71	1.18	7.0		0.120		
5.0	2.47	1.72	11.1		0.202		
5.2	3.38	2.48	17.4		0.302		
5.4	4.44	3.46	25.8		0.419	0.088	0.025
5.6	5.65	4.55	35.5		0.538	0.200	0.140
5.8	6.74	5.73	46.6		0.651	0.353	0.262
6.0	7.62	6.80	58.1		0.745	0.493	0.399
6.2	8.35	7.67	69.0		0.827	0.615	0.524
6.4	8.89	8.35	78.0		0.899	0.718	0.655
6.6	9.27	8.89	85.0		0.953	0.797	0.765
6.8		9.23	90.0		0.999	0.854	0.852
7.0			93.6	0.57	1.045	0.895	0.933
7.2				0.88	1.091	0.923	1.017
7.4				1.37	1.152	0.947	1.105
7.6				2.07	1.231	0.965	1.195
7.8				2.94	1.329	0.983	1.305
8.0				3.93	1.445	1.001	1.435
8.2				5.03	1.571	1.027	1.573
8.4				6.14	1.697	1.065	1.695
8.6	10.66			7.17	1.825	1.113	1.821
8.8	11.00	10.83	107.9	8.01	1.950	1.176	1.949
9.0	11.51	11.23	112.1	8.65	2.064	1.262	2.070
9.2	12.19	11.85	117.9	9.07	2.185	1.362	2.194
9.4	13.09	12.70	125.2	9.40	2.313	1.488	2.338
9.6	14.18	13.75	134.6		2.451	1.642	2.520
9.8	15.39	14.90	145.6		2.587	1.865	2.750
10.0	16.60	16.05	156.6		2.717		
10.2	17.68	17.09	166.6		2.832		
10.4	18.60	17.98	176.2		2.937		
10.6	19.35	18.74	184.4		3.042		
10.8		19.39	190.5		3.153		
11.0		19.94	195.2				

TABLE II

Relations Between pK′ and Ionic Strength for Piperazine Dihydrochloride at 25° C.

Ionic strength	pK_1'	Ionic strength	pK_2'
0.0100	5.44	0.0174	9.74
0.0238	5.49	0.0519	9.78
0.100	5.56	0.174	9.82
0.238	5.68	0.571	9.87
1.00	5.79	0.800	9.88
1.67	5.86		

5.32 and pK_2 of 9.70. Bredig (1894) found a second ionization constant of 6.4 $\times 10^{-5}$ at 25° C., but failed to report a first ionization constant. Kolthoff (1925, 1925) reported a pK_1' of 4.05 and a pK_2' of 8.34 for piperazine at 15° C. Since the temperature he used is different from that used in the present experiment, the results are not comparable. A search of the literature reveals no other reports of the ionization exponents of piperazine.

On the other hand, because of interest in amphoteric electrolytes and in dipeptides, numerous studies of the K_a, K_b, pK_1', pK_2', pK_1, and pK_2 values of glycylglycine have been made (Euler, 1907; Dernby, 1916, 1917; Eckweiler et al., 1921; Harris, 1923; Levene et al., 1924; Täufel and Wagner, 1927; Branch and Miyamoto, 1930; Mitchell and Greenstein, 1930; Fromageot and Watremez, 1930; Stiasny and Scotti, 1930; Greenstein, 1933; Johnson and Peterson, 1935; Neuberger, 1937; Konikov, 1938; Carr and Shutt, 1939; Glasstone and Hammel, 1941; Smith and Smith, 1942). Included above are numerous titration curves for glycylglycine in different media, but no actual tables for the preparation of buffers have been previously reported.

SUMMARY

The advantages of piperazine dihydrochloride and glycylglycine as buffers include the low toxicity, the lack of volatility, the solubility, the availability of the pure products, the convenience and accuracy of buffer preparation, and the lack of precipitation of calcium and magnesium salts from sea water below a pH of 9.9.

Table I indicates the preparation of solutions of known pH involving piperazine dihydrochloride, glycylglycine, and mixtures of these two in distilled water and in sea water at 25° C. Because the determinations were not made with the hydrogen electrode this table must be considered as being susceptible to small error, especially on the alkaline side of pH 9.

Table II shows the pK_1' and pK_2' values for piperazine dihydrochloride for several ionic strengths. Using the Debye-Hückel equation, the extrapolated pK values of piperazine at infinite dilution were found to be 5.32 for pK_1 and 9.70 for pK_2.

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